# Modulating Radiation Resistance: Novel Protection Paradigms Based on Defenses Against Ionizing Radiation in the Extremophile *Deinococcus radiodurans*

## IV. Abstract

The eubacterium *Deinococcus radiodurans* is extremely resistant to ionizing radiation (IR) and other DNA-damaging conditions that traditionally have been considered inhospitable to life, but the underlying cellular resistance mechanisms remain poorly defined. *D. radiodurans* contains 4-10 identical copies of its genome per cell, and when irradiated to a dose of 10 kGy generates >400 genomic double-strand break (DSB) DNA fragments per cell. Yet, this amount of DNA damage in *D. radiodurans* does not typically lead to cell death. With few exceptions, bioinformatic and experimental reports indicate that genome configuration and copynumber, and protection and repair functions of *D. radiodurans* do not have unique properties that are essential or prerequisite for expression of the extreme resistance phenotype.

The proposed research will build on recent work which shows that bacteria can sustain lethal levels of oxidative protein damage under conditions which elicit relatively little DNA damage, where resistance correlates with high intracellular Mn and low Fe concentrations. *In vivo*, high levels of protein oxidation occur in radiation sensitive cells which have low Mn/Fe ratios, but not in radiation resistant cells which have high Mn/Fe ratios. Our results support a strong mechanistic link between solution phase chemistry of Mn ions in *D. radiodurans* and protein protection. Therefore, a new hypothesis on the nature of cellular pathways connecting the formation of reactive oxygen species (ROS) with endpoint biological damage has been proposed: oxidative protein damage is key in determining survival of bacteria exposed to stressors such as IR, ultraviolet (UV) radiation and desiccation. Our finding that IR-induced protein damage, but not DNA damage, is quantifiably related to survival is important since it may come to affect models of radiation toxicity, approaches to control recovery from radiation injury, and the development of radioprotectors based on Mn-dependent scavengers of ROS.

Intracellular oxidative protein damage in a given environment appears to be a primary contributor to cell death as well as damage, and hence a sensitive indicator of a cell's ability to survive and withstand the detrimental effects associated with ROS induced under IR, UV and desiccation. Conventional and high-throughput proteomic approaches are suitable to measuring the degree of protein oxidation in cells, and we have shown that the level of protein oxidation in *D. radiodurans*, and resistance of cells exposed to IR can be controlled by modulating intracellular Mn redox-cycling processes. New research is proposed here to develop stress indicator-assays based on protein oxidation, with the goal of developing the scientific understanding needed to exploit mechanisms that Nature has already developed to protect everything from the basic building blocks like proteins, all the way up to the cell itself. The two specific aims of this proposal are: (1) Develop quantitative assays for oxidative protein damage as indicators for IR, UV and desiccation damage in prokaryotic and eukaryotic cells; and (2) Develop novel radioprotectors and antioxidants based on catalytic Mn(II,III) redox-cycling processes identified in *D. radiodurans*. The proposed research will be conducted at the Uniformed Services University of the Health Sciences in Bethesda, MD with Michael J. Daly as the principal investigator.